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# Arabinogalactans from *Mimosa tenuiflora* (Willd.) Poiret bark as active principles for wound-healing properties: Specific enhancement of dermal fibroblast activity and minor influence on HaCaT keratinocytes

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ABSTRACT

*Ethnopharmacological relevance:* Aqueous extracts from the bark of *Mimosa tenuiflora* (Willd.) Poirett (Mimosaceae), tradionally known as "tepescohuite", are widely used for wound-healing and burns in middle and South America. No pharmacological data are available on the influence of aqueous extracts and high molecular constituents on human skin cells.
*Materials and methods:* Tests were performed on human primary dermal fibroblasts and human HaCaT keratinocytes by quantification of mitochondrial activity (MTT, WST-1), proliferation (BrdU incorporation), necrosis (LDH) and gene expression profiling (RT-PCR).
*Results:* Water extract WE (10 and 100 μg/mL) expressed loss of cell viability and proliferation in dermal fibroblasts. Ethanol-precipitated compounds EPC (10 μg/mL), isolated from WE significantly stimulated mitochondrial activity and proliferation of dermal fibroblasts. Minor stimulation of human keratinocytes by EPC was found only at 100 μg/mL level. The differentiation behavior of keratinocytes was not influenced by EPC. EPC had no influence on the expression of specific proliferation and differentiation related genes so that the mode of action remains unclear. By bioactivity-guided fractionation two arabinogalactan-enriched fractions (F2, F3) were isolated from EPC and identified as the stimulating principles of EPC against fibroblasts.
*Conclusions:* A significant *in vitro* stimulation of dermal fibroblast activity and proliferation by arabinogalactans from *Mimosa tenuiflora* provides a rational for the traditional use of the bark material for wound healing.



## 1. Introduction

*Mimosa tenuiflora* (Willd.) Poiret, traditionally known as "tepescohuite", is a perennial indeciduous shrub of the family Mimosaceae, widely spread in Central and South America. The bark is used in folk medicine as a traditional remedy to treat skin burns and wounds and to prevent inflammation (Grether, 1988). Since the successful application to burn victims of several catastrophic incidents in the 1980 in Mexico, *Mimosa tenuiflora* bark got into strong focus of modern scientific investigation of skin treatment. According to traditional Mexican ethnomedicine the dried and powdered bark material is directly applied to the skin lesions (Sanchez-Leon and Yashte, 1991; Camargo-Ricalde et al., 1994; Camargo-Ricalde, 2000) or is used as aqueous extracts (personal communication Mexican people and healers to J.Z., 2006/2007). Until now physiological effects as mitogenic and anti-ulcer effects are mainly shown for extracts prepared with organic solvents (Villarreal et al., 1991; Rivera-Arce et al., 2007a,b), which are not fully representing the traditional use of aqueous extracts. However, minor information is available concerning bioactivity of isolated compounds (Anton et al., 1993). The high contents of saponins (Jiang et al., 1991) and tannins in the bark material are claimed to be responsible for potential wound-healing effects (Meckes-Lozoya et al., 1990), due to antimicrobial (Lozoya et al., 1989; Heinrich et al., 1992), anti-inflammatory (Villarreal et al., 1991) and cicatrizing effects (Rivera-Arce et al., 2007a,b). Because prestudies indicated that aqueous extracts from *Mimosa tenuiflora* bark contain higher amounts of polysaccharides (data not shown, unpublished results of the authors) the following studies were carried out to investigate the influence of these polymers on skin cell physiology besides traditionally used aqueous multi-component extracts.

Strongly stimulating effects of plant polysaccharides on human skin cell physiology have been shown for several times in the recent

*Abbreviations:* BrdU, bromodeoxyuridin; FCS, fetal calf serum; GPC, gel permeation chromatography; AEC, anion exchange chromatography; pNHAK, primary normal human adult keratinocytes (from dermal resectates); pNHDF, primary normal human dermal fibroblasts (from dermal resectates); EPC, ethanol-precipitated compounds; WE, water extract.

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years (Gloaguen et al., 2008; Deters et al., 2005a,b; Brunold et al., 2004). Especially the strong enhancement of cell viability and proliferation rates in human skin fibroblasts and keratinocytes suggest a positive impact of certain carbohydrates on the regeneration after skin lesions. With respect to these facts, the aim of the following study was to isolate, purify and characterize polysaccharides from a water extract of *Mimosa tenuiflora* bark and to investigate their impact on *in vitro* cultures of human skin cells.

## 2. Materials and methods

### 2.1. General experimentation procedure

If not stated otherwise all chemicals were purchased by VWR (Darmstadt, Germany).

The powdered bark of *Mimosa tenuiflora* was obtained from Yerbamex, Mexico (Lot. L/YGR3703305) and was identified by anatomical and phytochemical characteristics according to Rivera-Arce et al. (2007a,b) and by the comparison with reference drug material. A voucher species is deposited in the archives of IPBP, University of Münster, Germany (MT 2008/1 JZ).

### 2.2. Preparation of water extract, raw polysaccharide and polysaccharide fractions

Powdered bark (5 g) from *Mimosa tenuiflora* was extracted three times for 20 h with 20 g of water at 4 °C under strong stirring. The extract was filtered (Cellulose, Schleicher & Schuell, Dassel, Germany), concentrated under vacuo at a 4 °C not exceeding temperature and lyophilized (yield 12.5%, related to the starting bark material). This water extract was named WE.

For isolation of the polysaccharides powdered bark (50 °C) was extracted three times for 20 h with 200 g of water at 4 °C under strong stirring. The extract was filtered, concentrated under vacuo at a 40 °C not exceeding temperature to 200 mL which were precipitated into 800 mL of ice-cold ethanol 96%. The resulting precipitate was isolated by centrifugation (3000 × g, 10 min), dissolved in 10 mL water, dialyzed (Cellulose membranes, MWCO 3.5 kDa, Roth, Karlsruhe, Germany) and lyophilized to yield 0.4% of a slight reddish colored preparation, named ethanol-precipitated compounds (EPC).

Fractionation of EPC was achieved by anion exchange chromatography on DEAE Sephacel® (30 cm × 2.5 cm) (GE-HealthCare, Munich, Germany) in the phosphate form and elution by a step gradient of deionized water, followed by sodium phosphate buffers pH 6.0, ion strength 0.1, 0.25, 0.5, and 1 mol/L, flow rate 100 mL/h, fraction size 2 mL. Carbohydrate-containing fractions were pooled (see Fig. 1), concentrated under vacuum, dialyzed and lyophilized. GPC was achieved on Superose™6 (flow rate 1 mL/min) and Sepharose CL6B (flow rate 20 mL/min) with NaCl 0.1 mol/L as mobile phase.

### 2.3. Carbohydrate analysis

Total carbohydrates in AEC- and GPC-fractions were assayed using the resorcinol-sulphuric acid test (Monsigny et al., 1988). Determination of total uronic acids was performed according to the method of Blumenkrantz and Asboe-Hansen (1973), with *o*-hydroxydiphenyl in a modification for 96-well-microtiter plates using galacturonic acid as reference. Quantification of monomeric carbohydrates was accomplished by ion-exchange HPLC with pulsed-amperometric detection (Bio-LC, Dionex, Idstein, Germany) with AS50 auto sampler, GS50 gradient pump, AS50 oven and ED50 electrochemical detector on a CarboPac™ PA1 analytical column, 2 mm × 250 mm, CarboPac™ PA1, guard column 2 mm × 50 mm and BorateTrap™ Trap, 4 mm × 50 mm. Elution occurs by a gradient program using water and NaOH 0.1 M for neutral sugars, and ternary gradient water, NaOH 0.1 M and NaOAc 0.5 mM for uronic acids.

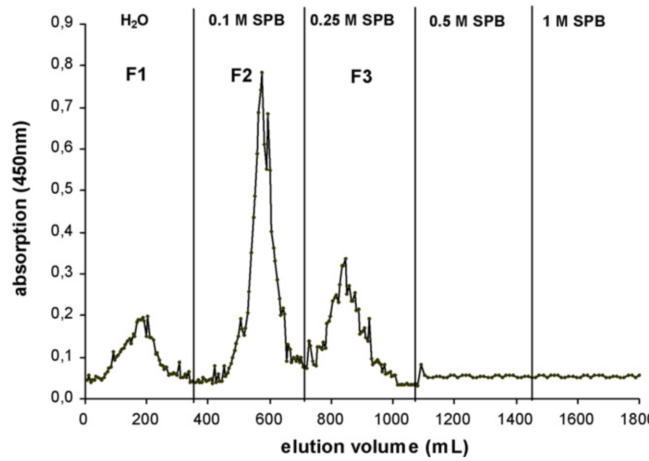


**Fig. 1.** Anion exchange chromatography of EPC from *Mimosa tenuiflora* on DEAE-Sephacel® using a step gradient of water, 0.1 M, 0.25 M, 0.5 M, and 1 M sodium phosphate buffer (SPB). Fraction size 2 mL.

Polysaccharides were hydrolyzed with 2N trifluoracetic acid at 121 °C for 1 h.

The determination of molecular weight distribution of polysaccharides was performed by FPLC (GE Healthcare, Munich, Germany) on a Superose™6 column using standard dextrans (Sigma, Steinheim, Germany) for calibration. Void volume was determined with DextranBlue.

Quantification of residual protein was performed according to the method of Bradford (1976) using standard bovine serum albumin BSA (PAA Laboratories GmbH, Austria) as reference compound.

Quantification of amino acid composition was accomplished by ion-exchange HPLC with pulsed-amperometric detection (Bio-LC, Dionex, Idstein, Germany) on a AminoPac™ PA1 analytical column, 2 mm × 250 mm, AminoPac™ guard column 2 mm × 50 mm and BorateTrap™ Trap, 4 × 50 mm, with a Dionex disposable electrode for amino acids®. Elution occurred by a gradient program using water NaOH 250 mM and NaOAc 1 M.

### 2.4. Methods of cell biology

HaCaT keratinocytes were kindly provided by Prof. Fusenig (DKFZ, Heidelberg, Germany). Primary keratinocytes and fibroblasts were obtained from surgical resectates (University Clinic of Münster, Germany, Department of Dermatology, Department of Pediatrics) of various Caucasian subjects and were cultivated either as pNHAK (primary normal human adult keratinocytes) or pNHDF (primary normal human dermal fibroblasts). Approvals of the studies were made by the local ethical committee of University of Münster (acceptance no. 2006-117-f-S).

Decontamination of skin and isolation of keratinocytes was carried out according to Deters et al. (2005a). For isolation and propagation of fibroblasts the dermis was washed with PBS and incubated for 2–3 weeks in cell culture flasks in fibroblast growth medium (MEM high glucose, FCS (10%) and L-glutamine (1%) (PAA; Pasching, Austria). Fibroblasts growing out from the tissue tend to form monolayers within the flask bottom, from which cells were isolated and used for further passages.

Permanent culture of HaCaT keratinocytes was performed in D-MEM high glucose medium supplemented with FCS (10%), penicillin/streptomycin (1%), glutamine (1%) and non-essential amino acids (1%) (PAA, Pasching, Austria).

Submerse cultivation of primary keratinocytes (pNHEK) and primary fibroblasts (pNHDF) was performed at 37 °C, 5% $CO_2$. HaCaT keratinocytes were cultivated at 35 °C, 5% $CO_2$.

**Table 1**
Investigated genes and related assay IDs analyzed via quantitative Real-Time PCR.

| Gene | Assay-ID | Gene | Assay-ID |
|---|---|---|---|
| EGF | HS01099990_m1 | KRT10 | HS00166289_m1 |
| SMAD3 | HS00706299_s1 | PLA | HS00179898_m1 |
| PKC-alpha | HS00176973_m1 | FN1 | HS00415006_m1 |
| 18s | HS99999901_s1 | Col1A2 | HS00164099_m1 |
| FGF7/KGF | HS00384281_m1 | EGFR | HS01076068_m1 |
| FGFR2/KGFR | HS00240796_m1 | HGF | HS00300159_m1 |
| STAT6 | HS00598618_m1 | TGF-β | HS99999918_m1 |
| InsR | HS00169631_m1 | IVL | HS00846307_s1 |

The investigations with pNHEK and pNHDF were carried out with cells from the 2nd–6th passage.

Before incubation with the test compounds cells were directly adapted to serum- and BPE-free medium (pNHEK: MCDB 153 complete, Biochrom, Berlin, Germany; pNHDF: MEM high glucose, SerEx® (10%) and L-glutamin (1%) (PAA, Pasching, Austria). *In vitro* testing of test compounds was performed concerning mitochondrial activity by MTT test (Mosmann, 1983), BrdU incorporation assay (Porstman et al., 1985), and differentiation assay (Deters et al., 2008). WST-1 test was performed according to the manufacturer's instructions (Roche, Penzberg, Germany). For details of cultivation, assays and procedures see (see Deters et al., 2005a,b, 2008).

### 2.5. Gene expression analysis (RT-PCR)

Gene expression studies were performed on pNHEK and pNHDF at a cell density of $5 \times 10^4$ cells/mL in 6-well microtiter plates in 2 mL of medium. After 24 h the medium was removed and substituted with PCR medium (pNHDF: MEM high glucose, 1% glutamine) (PAA, Pasching, Austria); pNHAK: MCDB 153-medium basal (Biochrom, Berlin, Germany). Incubation with test compounds was done for 24 h. Total mRNA was isolated using innuPREP RNA Mini Kit (Analytik Jena, Jena, Germany) according to the instructions of manufacturer. RNA content in the samples was calculated in comparison with calibrated agarose gels (1%) after ethidium bromide staining. Reverse transcription was performed by High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, USA). cDNA was diluted with RNAse-free water to 15 ng cDNA/9 μL. RT-Polymerase chain reaction (PCR) was performed with 1 μL of TaqMan gene expression assay (20×) including the primers, specified in Table 1 (Applied Biosystems, Foster City, USA), 10 μL TaqMan Universal MasterMix (2×, without Amperase) (Applied Biosystems, Foster City, USA) and 9 μL of the diluted target cDNA on a 7300 Real Time PCR (Applied Biosystems, Foster City, USA).

### 2.6. Statistics

Analysis of the influence of test compounds on cell physiology: statistical evaluation was performed after ANOVA by Dunnett-T post hoc test. *p*-Values $<0.05$ were considered significant. All data presented are the means of 6 random samples from one experiment, which was confirmed three times by independent recurrences (errors bars: ±SD).

## 3. Results

### 3.1. Analytical characterization of polysaccharide preparations

From *Mimosa tenuiflora* bark a cold water extract (WE) was prepared (yield 12.5%) from which an ethanol-precipitable polysaccharide preparation (EPC) was isolated (yield 0.4%) The use of cold water excluded the extraction of physiologically inactive starch, present in high amounts in the bark.

**Table 2**
Carbohydrate composition of EPC, F2 and F3 from *Mimosa tenuiflora* bark as determined by HPAEC-PAD after TFA hydrolysis against external standard calibration.

| Carbohydrate monomer | EPC [mol%] | F2 [mol%] | F3 [mol%] |
|---|---|---|---|
| Fucose | 1.6 | 1.7 | 2.9 |
| Rhamnose | 6.7 | 5.7 | 12.8 |
| Arabinose | 21.2 | 24.5 | 17.7 |
| Galactose | 24.6 | 37.8 | 22.5 |
| Glucose | 10.6 | 4.4 | 8.3 |
| Mannose | 16.4 | 16.6 | 11.6 |
| Xylose | 3.1 | 5.4 | 6.9 |
| Fructose | 1.0 | 0.0 | 0.0 |
| Ribose | 0.4 | 0.0 | 0.0 |
| Galacturonic acid[a] | 9.7 | 1.9 | 13.0 |
| Glucuronic acid[a] | 4.7 | 2.0 | 4.3 |

[a] Determined as C-6-reduced Gal/Glu.

EPC was further fractionated by anion exchange chromatography on DEAE-Sephacel® leading to three distinct fractions (Fig. 1). Neutral polysaccharides were eluted with water (26.6%, F1), acidic polymers eluted at 0.1 M sodium phosphate buffer (44.5%, F2) and at 0.25 M sodium phosphate buffer (28.9%, F3).

Analytical characterization of EPC and fractions F2 to F3 by HPAEC-PAD indicated a high content of arabinose, galactose, and mannose for EPC and F2 (Table 2). The acidic polymers of F3 were composed mainly by rhamnose, arabinose, and galactose, beside higher amounts of galacturonic acid. F1 was not investigated, because during because during assay tests this fraction was shown to be physiologically inactive (see Section 3.2).

The molecular weights of fractions F2 and F3 were shown to be heterodispers, eluting within gel permeation chromatography on Superose™6 over wide ranges. A further fractionation towards distinct subfraction was not possible. F2 had a molecular weight distribution from 5 to 140 kDa with a mean weight of 6.8 kDa. The molecular weight of F3 ranged from 5 up to 81 kDa, mean weight 6.8 kDa.

Significant protein amounts were determined in EPC (5.5%), F2 (3.0%) and F3 (1.2%).

The presence of an arabinogalactan protein (AGP) by precipitation with Yariv reagent (Yariv et al., 1962) using radial agarose diffusion test was clearly proven for F3. F2 did not contain any AGP. The EPC could not be tested because of its own reddish color, interfering with the Yariv assay.

HPAEC-PAD analysis of amino acid composition (Table 3) indicated F3 to be an untypical AGP without hydroxyproline (Showalter, 2001), but with unusual high amounts of glutamate (33%) and cystine (24%).

The protein part of F2 was, as expected, different from that of F3 and was shown to be mainly composed of glutamine, alanine and glutamate (Table 3).

### 3.2. In vitro assays

The water extract WE, EPC and fractions F1–F3 were investigated with respect to their physiological activity using primary normal human fibroblasts (pNHDF) and immortalized HaCaT keratinocytes, a non malignant cell-line. After 72 h incubation with the test fractions at 10 and 100 μg/mL, cell viability was investigated by measuring the cellular dehydrogenase activity by MTT and WST-1 test. The proliferation rate was determined by immunochemical detection of the incorporated thymidine-analogue BrdU into replicating DNA (Porstman et al., 1985).

The water extract WE caused a drastically decrease of cell viability (Fig. 2A) and proliferation rate (Fig. 2B) of primary fibroblasts in a dose-dependent manner at concentrations of 10 and 100 μg/mL. WE is therefore assessed to be strongly toxic to fibroblasts, probably due to the high amounts of polyphenols and saponins (Rivera-Arce

**Table 3**
Amino acid composition of EPC, F2 and F3 protein part from *Mimosa tenuiflora* bark as determined by HPAEC-PAD on AminoPac™ after hydrolysis with 6 M HCl, 110 °C, 6 h; Tryptophan was determined after hydrolysis with 4.25 M NaOH, 110 °C, 20 min.

| Amino acid | EPC [mol%] | F2 [mol%] | F3 [mol%] |
|---|---|---|---|
| Arginine | 0.4 | 0.6 | 6.3 |
| Lysine | 4.4 | 5.1 | 0.0 |
| Glutamine | 4.5 | 30.7 | 7.4 |
| Asparagine | 9.2 | 0.0 | 0.0 |
| Alanine | 8.7 | 9.2 | 7.7 |
| Threonine | 4.9 | 4.0 | 1.7 |
| Glycine | 8.7 | 4.8 | 8.1 |
| Valine | 5.0 | 2.8 | 0.0 |
| Hydroxyproline | 1.6 | 2.3 | 0.0 |
| Serine | 6.2 | 6.1 | 2.4 |
| Proline | 3.6 | 2.0 | 0.3 |
| Isoleucine | 4.4 | 0.0 | 2.9 |
| Leucine | 6.8 | 1.4 | 0.0 |
| Methionine | 0.6 | 3.5 | 0.0 |
| Histidine | 3.3 | 4.7 | 1.2 |
| Phenylalanine | 2.9 | 1.8 | 1.0 |
| Glutamate | 3.1 | 17.8 | 32.6 |
| Aspartate | 2.9 | 0.0 | 0.0 |
| Cystine | 7.4 | 0.0 | 24.0 |
| Tyrosine | 2.2 | 3.2 | 1.1 |
| Tryptophane | 9.4 | 0.0 | 3.4 |

et al., 2007a,b). It was surprising that WE did not change the dehydrogenase status and the proliferation rate of HaCaT keratinocytes (data not shown). Both cell types, fibroblasts and keratinocytes seem to be greatly different concerning their susceptibility towards the toxic principles of WE.

In contrast, the ethanol precipitable compounds from the water extract (EPC) induced a significant increase in cell viability and proliferation rate in fibroblasts at 10 μg/mL, whereas at 100 μg/mL no enhancement of dehydrogenase activity and proliferation was visible anymore. This non-dose dependency is assessed to be due to the presence of potential inhibiting polyphenolic compounds in the EPC, which were visible by the slightly reddish color of EPC.

Investigation of EPC on HaCaT keratinocytes did not indicate any stimulating effect at 10 μg/mL level, while at 100 μg/mL a slight, but significant stimulation of mitochondrial activity and proliferation was observed (data not shown).

Bioassay-guided fractionation of EPC by anion exchange chromatography towards the subfractions F1–F3 revealed no physiological activity of F1 (data not shown), while F2 and F3 clearly stimulated the cellular dehydrogenase activity at 10 and 100 μg/mL significantly (Fig. 3A). Furthermore F2 and F3 triggered fibroblasts into a higher mitogenic status (Fig. 3B).

As expected, F2 and F3 as purified subfractions of the EPC did not influence the mitogenic status and cellular dehydrogenase activity of HaCaT keratinocytes (data not shown).

In further investigations EPC was tested concerning to its ability to influence the differentiation behavior of primary normal human keratinocytes (pNHAK), an indispensible competence to rearrange the physiological barrier of the skin and pivotal process of wound healing. As indispensable cytomarkers for differentiation within primary keratinocytes involucrin and cytokeratins K1 and K10 were monitored by semi-quantitative dot-blot-technique using specific antibodies. After a 6-day incubation of pNHAK with EPC (10 μg/mL) no enhanced formation of these markers compared to the untreated control was shown, while the calcium-treated positive control was clearly differentiated. Therefore, EPC is assessed to have no influence on the differentiation status of keratinocytes.

To gain more information on the mode of action of EPC, the influence on the gene expression of proliferation and differentiation related specific growth factors, their receptors and intracellular signal molecules in human primary fibroblasts and keratinocytes was investigated (Table 4). After a 24 h incubation of human dermal fibroblasts and primary keratinocytes with EPC (10 μg/mL) quantitative two step Real Time PCR was performed. Interestingly, EPC did not influence any expression of the investigated genes to a significant extend (Table 4). This is in strong contrast to other polysaccharides investigated recently (Deters et al., 2005a,b), which have been shown to interact with extra-membranous receptors, followed by an initiation of signal transduction processes towards a higher gene expression. From these results *Mimosa tenuiflora* polysaccharides are not acting via the "usual" skin cell activation cascades.

## 4. Discussion

The bark of *Mimosa tenuiflora* is a traditional remedy for several skin ailments like burns, ulcer and psoriasis and plays furthermore a role in the treatment of wounds (Rätsch, 1998). For ethnopharmaceutical use the bark is usually powdered and often applied as decoct or cataplasm (personal communication of Mexican healers and physicians to J.Z., 2006–2007).

According to the studies performed with *Mimosa tenuiflora* to the present, it seems that the wound-healing activity of this plant is due to a combination of the several different compounds (the bark contains tannins, saponins, an alkaloid fraction, lipids,

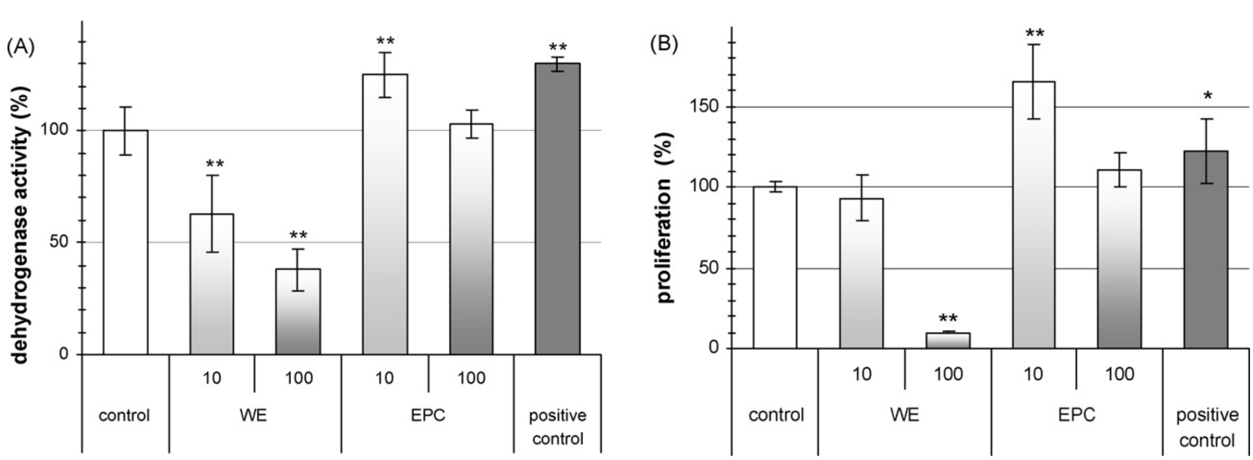


**Fig. 2.** Dehydrogenase activity (A) and proliferation rate (B) of primary natural human fibroblasts (pNHDF) incubated with water extract (WE) and ethanol-precipitated compounds (EPC) from *Mimosa tenuiflora* at 10 and 100 μg/mL over a 48 h incubation period. Bars represent standard deviation (SD) with $n = 6$ with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the untreated control group. Positive control 1% FCS; negative control untreated cells in medium without FCS.

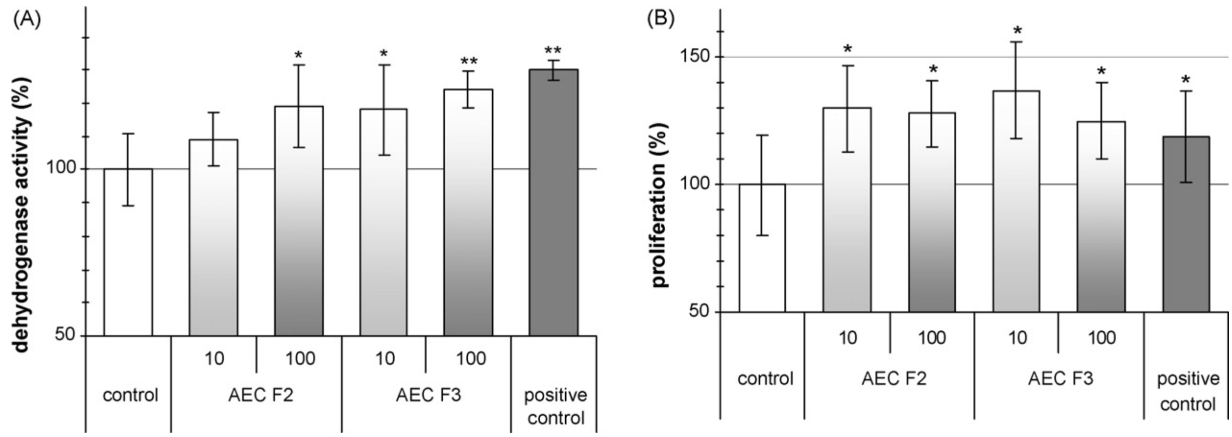


**Fig. 3.** Dehydrogenase activity (A) and proliferation rate (B) of primary natural human fibroblasts (pNHDF) incubated with AEC fractions F2 and F3 from *Mimosa tenuiflora* at 10 and 100 μg/mL over a 48 h incubation period. Bars represent standard deviation (SD) with $n = 6$ replicates with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the untreated control group. Positive control 1% FCS; negative control untreated cells in medium without FCS.

**Table 4**
Typical growth factors, growth factor receptors, signal proteins and marker proteins from keratinocytes (pNHEK) and dermal fibroblasts (pNHDF) investigated by RT-PCR on gene expression level after treatment of cells with EPC 10 μg/mL after 24 h of incubation time. (+) Factors expressed.

| | Functionality | Proliferation | Differentiation | Occurrence in pNHDF | Occurrence in pNHEK | Relative expression |
|---|---|---|---|---|---|---|
| EGF | Epidermal growth factor | + | + | + | + | <1 |
| EGF-R | EGF receptor | + | + | + | + | <1 |
| KGF | Keratinocyte growth factor | + | | + | | <1 |
| KGF-R | KGF receptor | + | | | + | <1 |
| HGF | Hepatocyte growth factor | + | | + | + | <1 |
| Ins-R | Insulin receptor | + | | + | + | <1 |
| TGF-β | Transforming growth factor | | + | + | + | <1 |
| FN 1 | Marker protein maturation | + | | + | | <1 |
| Collagen 1A2 | Marker protein maturation | + | | + | | <1 |
| Keratin 10 | Marker protein differentiation | | + | | + | <1 |
| Involucrin | Marker protein differentiation | | + | | + | <1 |
| STAT 6 | Signal proteins of IL-4 signal transduction | + | | + | + | <1 |
| PRKC A | Signal protein of $Ca^{2+}$ signal transduction | | + | | + | <1 |
| PLA2 | Signal protein of $Ca^{2+}$ signal transduction | | + | | + | <1 |
| Smad 3 | Signal protein of TGF-β signal transduction | | + | | + | <1 |

phytosterols, monomenric and polymeric carbohydrates, lupeol, methoxychalcones, and kukulkanins,) some of them with cytotoxic and antimicrobial activity (saponins, tannins), and different pharmacological activities.

For this reason, especially the water soluble compounds of this herbal material were in the focus of interest.

It was interesting to find strong cellular toxicity of the water extract WE against *in vitro* cultured skin fibroblasts, while HaCaT keratinocytes were not influenced by WE. The bark of *Mimosa tenuiflora* contains high amounts of saponins and polyphenolic compounds (Rivera-Arce et al., 2007b). The potential cell toxicity may be related to the presence of polyphenols as described (Balasubramanian and Eckert, 2007). On the other side, these compounds seem to play an important role in the antimicrobial activity of *Mimosa tenuiflora* at the surface of the skin (Meckes-Lozoya et al., 1990; Heinrich et al., 1992). Also within treatment of severe skin ulcer the positive effects of *Mimosa tenuiflora* extracts were clearly related to the high content of polyphenols with a high cicatrization potential (Rivera-Arce et al., 2007a).

The greatly different susceptibility of fibroblasts and HaCaT keratinocytes towards the toxic principles of WE may be due to the different functions of these two cell types in the skin, leading to variations in metabolism and sensibility. Further it has to be pointed out that primary cells were used in the case of fibroblasts, while HaCaT keratinocytes originated from an immortalized cell line. Differences in cell metabolism regarding to this fact were shown in recently published data (Schürer et al., 1993; Törmä et al., 1999).

In contrast to the cell toxic effects of WE, the high-molecular material EPC, isolated by ethanol precipitation from WE, showed a strong potential to enhance the viability and proliferation of dermal fibroblasts and at higher concentration also on HaCaT keratinocytes.

The EPC still contains minor amounts of residual polyphenols. A toxic potential of these residual compounds on fibroblasts can explain why EPC induces no enhanced cell viability and proliferation at higher concentrations. At 10 μg/mL the amounts of polyphenols in the EPC seem to be to low for a toxic effect, so the stimulating activity of the polysaccharides prevails. At 100 μg/mL this stimulating effect is compensated by the toxic effect of the polyphenols. This hypothesis was proven by a complete removal of polyphenols from EPC by anion exchange chromatography on DEAE-Sephacel®, a material well known to interact strongly with polyphenols. As expected, the purified polysaccharides did not exhibit any cell toxicity.

EPC was fractionated into an untypical arabinogalactan protein (AGP, F3) and an arabinogalactan polysaccharide not reacting as AGP (F2). Both polymers were potent stimulators of dehydrogenase activity and proliferation of skin fibroblasts. Another polysaccharide fraction (F1) obtained from EPC was inactive. This suggests that the arabinogalactan-carbohydrate structure plays an important role in physiological activity of the *Mimosa tenuiflora* within wound healing. The protein part of both polymers F2 and F3 does not seem to contribute to a major extent to the stimulating activity.

The cellular differentiation of keratinocytes is an indispensable process of building up the *stratum corneum*, the physiological bar-

rier of the skin, especially during reepithelialisation of injured skin in the process of wound healing. So it was surprising that the EPC did not show any enhancing effect on differentiation of *in vitro* cultured primary keratinocytes. Furthermore, gene expression analysis provided no information about an influence of EPC on the gene expression of factors typically described for regulation of proliferation and differentiation of keratinocytes and skin fibroblasts. So the mode of action of the EPC remains unclear.

On the other side, fibroblasts react with a strong improvement of viability and proliferation in the presence of arabinogalactan polymers, indicating that the primary cellular target of *Mimosa tenuiflora* compounds are the fibroblasts within the connective tissue. Such a strong stimulation of fibroblasts can be assessed as a powerful tool for initiating wound-closure and production of filling and extracellular material within wounds. From our points of view this is the first report that plant secondary products specifically interact with different cell types in skin architecture.

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